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The Role of Human Papillomavirus in Head and Neck Cancer

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CHAPTER

Summary
and future perspectives

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SUMMARY

Over the last three decades, it has become clear that infection with high-risk human papillomavirus (HPV) is etiologically linked to the development of head and neck squamous cell carcinomas (HNSCCs), particularly those carcinomas that arise in the oropharyngeal region. Prevalence rates of HPV-positive oropharyngeal squamous cell carcinomas (OPSCCs) vary between studies, ranging from 20% to 90%. This may be related in part to the lack of a standardized HPV detection assay as well as to the time period in which HPV prevalence is investigated, as rising incidence rates have been reported over the last decades. (1-4) In **Chapter 2**, we validated our previously defined test algorithm for HPV detection in formalin-fixed paraffin-embedded tumor specimen. This test algorithm consists of a p16^{INK4A}-immunohistochemistry (p16-IHC) followed by a HPV DNA PCR on the p16-immunopositive cases. The test algorithm showed an accuracy of 98%. A standardized HPV detection method is urgently awaited and we encourage to use this validated algorithm for HPV detection.

Using the validated algorithm we found a significant increase in the proportion of HPV-positive OPSCCs, from 5.1% in 1990 to 29.0% in 2010. However, the increase in the proportion of HPV-positive OPSCC has only been observed at our institute and cannot be automatically extrapolated to the whole Dutch population. Future research should focus on rising prevalence rates of OPSCCs in the Netherlands and in particular the HPV-positive OPSCCs.

HPV-associated oropharyngeal carcinomas are considered to be a different tumor entity, based on biological, epidemiological and clinical differences, when compared to the HPV-negative OPSCCs. The most important clinical difference between patients with HPV-positive and HPV-negative OPSCCs is related to the prognosis. Several retrospective and prospective studies in the United States, Australia and Western Europe have consistently demonstrated that HPV-positive OPSCC is associated with a more favorable prognosis. (5-10) However, a common limitation of most studies is the selection of the studied population. As most of these studies are based on protocol-driven trials, with well-defined inclusion and exclusion criteria, there is a selection bias tendency for younger and hence healthier patients. Additionally, most clinical trials focus on patients with advanced stage of disease who are treated by chemoradiation. Patients with stage I/II disease are treated differently and hence little data on this group is available. In **Chapter 3** we determined HPV prevalence in a large cohort of 841 patients who were diagnosed with an OPSCC between 2000-2006 in two main university hospitals in the Netherlands. Subsequently, the correlation between survival and HPV-status was determined in the patients who were curatively treated (723 out of 841). Patients with an HPV-positive OPSCC had a more favorable overall survival

(73.5% vs. 40.9% after five years) compared to patients with an HPV-negative OPSCC. Next, a prognostic model was developed, based on this large cohort of patients. This model revealed that the main prognostic factor in patients with an OPSCC is HPV-status. In HPV-positive patients, nodal stage did not influence the prognosis. Instead, comorbidity was the most important prognostic factor. This new model is applicable for a more general group of patients and not only for patients included in clinical trials. We propose that the current TNM classification for patients with an OPSCC is insufficient for patients with HPV-positive tumors, as nodal stage seems not to be of prognostic value for these patients. Therefore, we encourage incorporating HPV-status into the next edition of the TNM classification.

As patients with HPV-positive OPSCCs have a favorable prognosis, an opportunity now exists to investigate less intense treatment strategies for these patients. These treatment strategies should not compromise survival outcomes but lower the risk of potentially debilitating late effects of treatment. For the most part, patients with HPV-positive OPSCC are young and in good health. Thus, provision of a high level of quality of life and the fewest treatment complications are important considerations.

At this moment, trials are running to investigate de-escalation of therapy for patients with an HPV-positive tumor. The clinical consequences may be severe in case of a false-positive HPV test. (11) Therefore, a reliable HPV detection method, that allows evaluation of active, oncogenic HPV involvement in OPSCC, is of major importance for the selection of patients in these trials. However, at this moment, eligibility for randomization only involves a positive p16-immunostaining.

In **Chapter 3**, we determined the survival of patients with a p16-positive but HPV DNA negative OPSCC. The survival of patients with a p16-positive but HPV DNA-negative OPSCC was almost identical to the survival of patients with a 'truly' HPV-negative (negative for p16-immunostaining) OPSCC. In **Chapter 4**, we analyzed the genetic profile (LOH patterns) of these 'discordant (i.e. p16-positive but HPV DNA-negative) tumors'. The LOH patterns were not statistically different from those found in the HPV-negative tumors. LOH in the discordant group occurred at a high frequency and, according to the criteria set after comparing truly HPV-positive and truly HPV-negative cases, nine of ten discordant samples were classified as being HPV-negative. Thus, our studies categorize p16-positive but HPV DNA-negative OPSCCs as HPV-negative tumors based on survival patterns and genetic profiling. This implies that some patients participating in the current clinical de-escalation trials (in which eligibility for randomization only involves positivity on p16-immunostaining) might be inaccurately assigned as having an HPV-positive OPSCC and should be excluded. We emphasize the importance of performing HPV testing in addition to p16-IHC for proper identification of HPV-associated OPSCCs.

In the future, it would be interesting to further investigate the mechanism for p16 overexpression in these 'discordant tumors' that lack HPV DNA. There might be specific mutations or deletions in genes explaining the overexpression of p16 in this group of tumors. Realizing that p16 is frequently inactivated by mutations, homozygous deletions and promoter methylation in HPV-negative OPSCC does raise the question why it is activated in this particular subgroup of tumors. (12-14) Most logical explanation for p16-overexpression independent of HPV, could be a disruption of the pRb pathway by other as yet unidentified molecular mechanisms, such as activating CDK4 or CDK6 mutations combined with a premature senescence response of the cells.

Several theories have already been proposed to explain the improved outcome and response to therapy of HPV-positive patients. One of these hypotheses is the possible absence of field cancerization.(15) In **Chapter 5**, we further investigated this possibility. Since HPV is involved in the early stage of carcinogenesis in OPSCCs, its presence is considered a reliable marker for 'field cancerization'. We demonstrated that transcriptional active HPV is not present in the fields surrounding an HPV-positive OPSCC. The absence of this field cancerization effect might be a possible explanation for the fact that HPV-positive OPSCC patients develop less local-regional recurrences and SPTs and therefore might contribute to an improved outcome in these patients. A second hypothesis for the better outcome in HPV-positive patients, includes a higher sensitivity of the HPV-positive OPSCCs to radiation or chemoradiation. As previous studies have shown that a possible explanation for (chemo) radiation failure in HNSCC patients could be therapy resistance of cancer stem cells (CSCs), we aimed to further evaluate the levels of CSCs in HPV-positive patients and a possible correlation with survival. In **Chapter 6**, we analyzed the presence of stem cell markers CD44 and CD98 in the previously described cohort of patients that were curatively treated for an oropharyngeal tumor in the period 2000-2006. Sufficient FFPE material was available for 711 of these 723 patients. We showed that patients with an HPV-positive OPSCC have lower expression of the cancer stem cell markers CD44 and CD98 than patients with an HPV-negative OPSCC. Within HPV-positive patients a high fraction of CD98-positive tumor cells was associated with a significantly worse prognosis. These data suggest that the lower number of CSCs in HPV-positive tumors, might be a reason for a better response to (chemo) radiation. Furthermore, CD98-expression could be useful as a prognostic marker for treatment outcome. A third hypothesis for the favorable prognosis of patients with an HPV-positive OPSCC, includes a possible role of the host immune system in defeating the tumor. The relationship between the immune system, HPV status and outcome remains an interesting area of future research.

FUTURE PERSPECTIVES

Screening options for HPV associated OPSCC

Death rates from cervical cancer have dramatically fallen in populations that have access to cervical cytology screening. (16;17) By contrast, there are no widely utilized and validated screening methods for OPSCC. The cervical transformation zone, where most cervical cancers arise, is relatively easily accessible to visual or colposcopic inspection and direct cell and tissue sampling. In contrast, the situation in head and neck carcinogenesis is different as the majority of HPV-positive cancers arise from the invaginating tonsillar crypt epithelium and possible preneoplastic changes are not easily visible on the surface epithelium. Moreover, the absence of field cancerization in HPV-positive OPSCCs, indicates that large, preneoplastic changes in the fields surrounding these tumors seem to be absent. A recent study evaluated whether screening with an equivalent of the Pap test allowed identification of HPV-induced oropharyngeal precancerous lesions; although a strong association was observed between HPV16 and cancer in visible lesions, there was no association between HPV16 and cytopathology in the absence of visible lesions. (18) An oropharyngeal Pap-test equivalent may not be feasible, likely due to limitations in sampling the relevant tonsillar crypt epithelium. Thus, the infeasibility of performing natural history studies of the histopathological progression of HPV-positive oropharynx in healthy subjects, limits studies for secondary prevention of oropharyngeal cancer. (19) In the absence of any clinically identifiable premalignant lesion, it seems that any future attempt for screening would rely on molecular biomarkers for premalignant disease.

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Another screening option for HPV-associated OPSCC, could be detection of oral HPV infection. Recent studies have highlighted the increased risk of OPSCC if HPV16 infection is detected in saliva.(20) HPV acquisition appears to increase around sexual debut with prevalent oral HPV detected in 1.5% of 12–15 year old and 3.3% of 16–20 year old individuals. (21;22) Oral HPV prevalence is higher among adults and is detected in ~ 4.5% of healthy adults.(23) Higher oral HPV prevalence has been reported in women with cervical HPV infection (24;25) and people infected with human immunodeficiency virus (HIV). (24;26) Oral HPV16 prevalence (the HPV type responsible for > 85% of all HPV-associated HNSCC) is found in 1.3% of healthy adults. (23) However, the great majority of these cases do not show progression to malignancy. Initial oral HPV natural history studies suggest that oral HPV persistence is similar to that known for anogenital HPV infection and that most prevalent infections clear spontaneously within a year. More longitudinal research is needed to better understand transmission of oral HPV infections, how likely infections are to be cleared, and what factors are associated with persistence.

Prophylactic HPV vaccination

The fact that detection of precancerous oropharyngeal lesions is still not possible, highlights the opportunity for primary prevention through prophylactic HPV vaccination, if proven efficacious and cost-effective. Prospective, randomized controlled trials have shown that HPV virus-like particle vaccines have very high efficacy in the prevention of high-grade cervical dysplasias caused by HPV16 and 18 infection in HPV-negative subjects. (27;28) At present, two HPV vaccines are available. The quadrivalent vaccine, Gardasil® (HPV4), protects against infection with HPV types -6, -11, -16 and 18. This vaccine was first licensed in 2006 for use in females aged 9–26 years old for the prevention of cervical, vaginal and vulvar cancers. More recent work has shown the vaccine to effectively prevent anal pre-cancers, thus the clinical indications for the vaccine have further expanded to include anal cancer prevention (29)

The second HPV vaccine, Cervarix®(HPV2), is a bivalent vaccine that provides protection against HPV types -16 and -18. This vaccine was licensed for use in the U.S. in 2009 for the prevention of cervical cancers (U.S. Food and Drug Administration (FDA), 2009). HPV2 was not tested in clinical trials for efficacy against vaginal, vulvar or anal cancers/pre-cancers. Therefore, these diseases cannot be included as approved clinical indications for the vaccine.

The clinical trials for both HPV vaccines were designed to evaluate vaccine efficacy for cervical and/or other anogenital “pre-cancers” as the clinical endpoints. The progression of cervical cancer along several defined precancerous states is well accepted. (30) This progression, combined with the generally long lag time between HPV infection and cervical cancer makes cervical pre-cancer a reasonable endpoint to assess in clinical trials. (31) In contrast, the typical progression between pre-cancerous and cancerous states for OPSCC is significantly less well established, though still highly biologically plausible. (13) The lack of well-defined precancerous lesions (i.e. the disease endpoint recommended by U.S. FDA), combined with lack of data regarding clearance of oral HPV infection has hampered the design of clinical trials to evaluate the efficacy of HPV vaccines for prevention of oral HPV infection. It is unclear whether the FDA and other regulatory agencies will accept virological endpoints of efficacy (i.e. persistent HPV infection). Such acceptance would greatly facilitate and accelerate the evaluation of current and future HPV vaccines for oropharyngeal cancer and cancer at other anatomical sites. (19) Assuming efficacy against oral HPV infections equivalent to that for cervical infection, a higher proportion of HPV-caused OPSCCs might be prevented with current generation vaccines, as the attributable fraction for HPV16 and 18 is 90-95%. An important consideration for the potential of HPV vaccines to reduce the incidence of OPSCC, is related to the rates of vaccine coverage in girls. Vaccination against HPV has been included in the national Vaccination Programme of the

Netherlands for 12-year-old girls since 2010. However, vaccination coverage for the birth cohort of 1997 was only 56%. (32) Vaccination coverage among girls must be sufficiently high (>80%) to sufficiently prevent transmission of oral HPV16 infection to boys through herd immunity. Populations with vaccination rates sufficient for herd immunity might observe reductions in OPSCC incidence as a consequence of reduced rates of genital-to-oral HPV transmission. However, in populations where vaccination coverage is below 80%, vaccination of boys is likely to be cost-effective.

Future direction of treatment for HPV-positive OPSCC

We can conclude that HPV-positive oropharyngeal cancer is a distinct subset of HNSCC with a favorable outcome. In future clinical trials, researchers will, at the very least, need to stratify for HPV-status. An opportunity now exists to investigate less intense treatment strategies that do not compromise survival outcomes but lower the risk of potentially debilitating late effects. Currently, several de-escalation trials are running. In the Eastern Cooperative Oncology Group (ECOG) E1308 trial (phase II trial), HPV-positive patients with locally advanced disease receive induction chemotherapy with cisplatin, paclitaxel and cetuximab (Erbix, Imclone). For those patients with a clinical complete response at the primary site, the definitive therapy of cetuximab and radiation is given to a lower total radiation dose of 54 Gy. The main aim of this study is to assess potential for a lower dose of radiation to control disease and to investigate toxic effects and quality of life variables.

A second trial is the Radiation Therapy Oncology Group (RTOG) 1016 trial (phase III trial). This study divides patients with an HPV-positive OPSCC in two arms: The control arm is accelerated radiotherapy (RT), 70 Gy in 6 weeks plus concurrent high dose cisplatin for 2 cycles. This will be compared to accelerated RT (70 Gy in 6 weeks) plus weekly cetuximab (8 doses in total). The aim of this study is to assess a potential for cetuximab (a molecular target agent), which is a less toxic alternative to concurrent chemoradiation.

Another phase III trial that compares concurrent chemoradiation versus RT plus cetuximab, is the DeESCALATE- HPV (Determination of Epidermal growth factor receptor inhibitor versus Standard Chemotherapy early And Late Toxicity Events in Human Papillomavirus positive oropharyngeal squamous cell carcinoma) trial. The control arm in this study is three doses of cisplatin (100mg/m²) at days 1, 22 and 43 from start of radiotherapy. This will be compared to one dose of cetuximab (400mg/m²) one week before start of radiotherapy, followed by a weekly dose of cetuximab (250mg/m², 7 doses in total) during radiotherapy.

In the future, these (and other) clinical trials should demonstrate whether de-intensification of treatment might be possible for patients with an HPV-positive OPSCC.

In conclusion, to increase our understanding of the etiology of OPSCC and HPV infection, specific areas for future research are recommended. Natural history studies of oral HPV infection would be necessary for the development of primary (prophylactic HPV vaccination) and secondary prevention (screening for HPV-induced OPSCCs) strategies analogous to those for cervical cancer. Regarding treatment of OPSCCs, we have to await the results of clinical trials running at the moment that consider reduction in the intensity of treatment.

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